THE CASE OF TRICHOMONAS[1]

DR. PHILIP HADLEY

THE great group of flagellated protozoa has, within the past two decades, afforded a wealth of interest for those concerned with pathogenic protozoology; and only in slightly lesser degree for those concerned with taxonomic problems involving these highly interesting microorganisms. The field of trypanosome research has, in itself, afforded much new data on morphology and on complicated life histories; and has been the chief center of interest for many years.

But there exists another group of the flagellated protozoa, represented by some of the commonest forms encountered in the intestinal tract of man and the lower animals, whose frequency of occurrence, simplicity of organization and freedom from imputations of possessing pathogenic powers, have enabled them to go their way, for the most part unmolested by the protozoologist. If the protozoan would escape the inquiring gaze of the researcher he must be self-effacing; he must lead a quiet life of seclusion, free from those public manifestations of unrest and mob movement which are sure to bring him, sooner or later, before the bar of investigation, whereupon his whole life is laid bare.

Trichomonas was such a quiet law-abiding protozoan before the trouble began, before he was detected in instigating internal revolutions which bid fair to annihilate the turkey-raising industry of the country. The circumstantial evidence which has been brought forward against him has served to reveal many aspects of the life history of *Trichomonas* with which we were not previously acquainted; to disclose his participation in activities for which he was previously regarded as scarcely capable, and to demonstrate the existence of certain family resemblances to some of his companions in mischief who have long been recognized as trouble-makers in the cell organizations of many animals.

Trichomonas is found living in the intestinal contents of nearly all animals and has, since its discovery by Donné in 1837, appeared under many different names. It is a small organism, built on an oval or pear-shaped plan, and measuring in the adult trophozoite stage, about $10\,\mu$ in length by 5 to $6\,\mu$ in breadth. The youngest free-swimming stages are much smaller, about $5\,\mu$ in length; and sometimes trophozoites are encountered that measure 12 or $13\,\mu$. Although usually of an elongate oval or pear shape, the morphology of the trophozoites is highly variable, and triangular or crescentic forms are frequently encountered, especially among the young. The anterior end is usually blunt, while the posterior end is frequently drawn out into a point.

If one adds to the salt solution in which these flagellates are being examined a little albumen or glycerin, to lessen the rapid swimming of the organism, some of the details of structure can be made out. The body plasm shows a greenish tint, and the nucleus, which is situated anteriorly, appears pinkish. In fresh preparations, one of the most obvious features is the axostyle, a short bristle-like structure which projects outward somewhere in the posterior quarter of the body, and which is seen, upon careful focusing, to extend into the body of the flagellate, running anteriorly to terminate somewhere in the vicinity of the nucleus (Figs. 1, 2). Inside the body the axostyle appears homogeneous in structure and bandlike.

Next to the axostyle, the most obvious feature is the vibratory or undulatory membrane which extends like a curved fin down the dorsal side of the flagellate body (Fig. 1). It is shallow at the beginning and at the end, but midway of its length it may have a depth of 2 to $3\,\mu$. Over this membrane may be seen to travel at 3 to $4\,\mu$ intervals, waves of motion from the anterior toward the posterior end of the body. If one follows closely the course of this membrane, it is found to have its origin in a granule, or in a group of small granules, located in front of the nucleus at the most anterior part of the animal, and known as the blepharoplast-complex. The granules

are very small, measuring not more than 0.5 to 1.0 μ, and stain deeply with the chromatin stains. Their function is at the present time only a matter of speculation. Tracing the dorsal membrane posteriorly, it is found to extend to the extreme end of the body, where it narrows and is continued in the form of a terminal flagellum ("Schlepp-geissel") which has a length ordinarily about equal to that of the body.

From the anterior end of the flagellate extend three more flagella (Fig. 1). These may be even longer than the body of the flagellate itself, and beat downward, as indicated by the arrow in Fig. 1. It frequently appears as if two of these three flagella were united in a common stalk at their base, so that they beat together, while the third flagellum beats independently. The origin of these three flagella is difficult to make out, but in many cases they appear to arise from one of the granules of the blepharoplast-complex, and usually not from the granule which is the origin of the undulatory membrane.

The only other structures which can be seen well in fresh preparations are the mouth or cytostome and the food vacuole. The cytostome is a horn-shaped opening which extends into the body on the ventral side, and just behind the nucleus (Fig. 2). It may be bordered by cilia. The beat of the anterior flagella is in such a direction that currents of fluid containing the bacteria which serve as the chief food for the flagellates, are driven into the mouth opening. Posterior to the nucleus, usually in about the middle of the cell body lies an oval space, the food vacuole (Fig. 2). It may sometimes be represented by a group of smaller vacuoles which coalesce to form a single cavity. In these vacuoles are usually present bacteria and cocci undergoing digestion. The structures mentioned above can be seen well in unstained organisms, but there are others which appear to advantage only upon staining. In preparations stained by the Heidenhain iron-hematoxylin method (wet process) the most noteworthy of the remaining structures is the chromatic line. This is a heavily-staining band which extends like the arc

of a circle from the blepharoplast to the point where the undulating membrane terminates. It thus follows closely, in the body plasm, the trend of the membrane, and is regarded as representing a kind of supporting structure. The chromatin line is heavier in its mid part and tapers at each end. As will be pointed out later, when in the process of spore formation, the trophozoites round off, the chromatin line becomes bent into a hoop, so that its extremities come very near to meeting (Fig. 4).

Another structure which appears with distinctness in stained preparations is the line of chromatic blocks (Figs. 3, 4). These peculiar bodies appear as a single or double row, or as a somewhat irregular line, of deeply staining granules extending from the region of the blepharoplast backward through the plasm to end somewhere in the posterior quarter of the cell. The anterior portion is likely to be thicker and sometimes may partially obscure the nucleus. The curve followed by the line of blocks is about parallel to that of the chromatic line and the two are seldom far distant from one another.

It is interesting to observe in connection with all of these structures that, in their arrangement, they produce in the flagellate organism a more or less perfect bilateral symmetry. The normal swimming position of the trophozoite is with the undulatory membrane above. Directly below this extends the chromatic line and below the chromatic line is the "line of blocks." The cytostome is in the midline and somewhat ventral. The blepharoplast is in the midline except in some of the stages of division. The food vacuoles occupy a variable position, but are usually grouped near the middle of the posterior body and caudad of the chromatic line. Sometimes it appears as if the line of blocks and the axostyle passed through the food vacuoles. The axostyle projects from the cell body in the midline although not necessarily at the most posterior part of the body. This symmetry is easily seen when the organisms are observed swimming freely in a favorable medium. Owing to the fact that the dorso-ventral diameter is greater than the transverse diameter, most
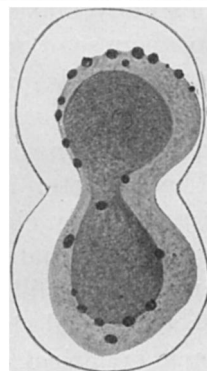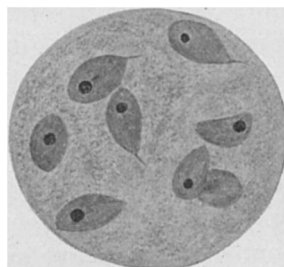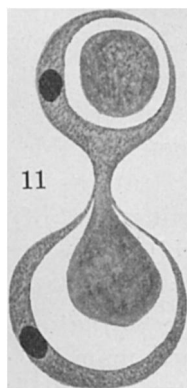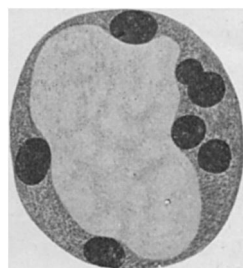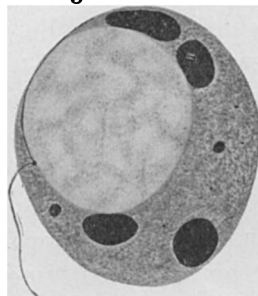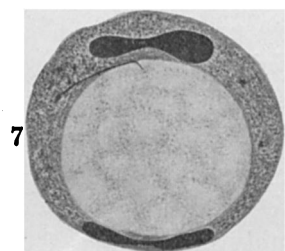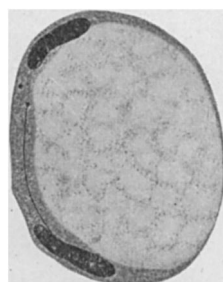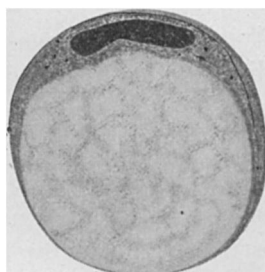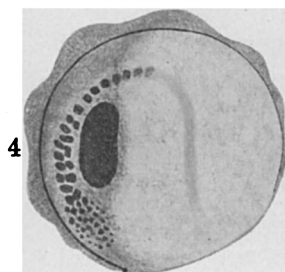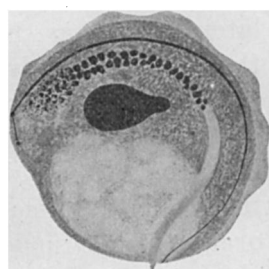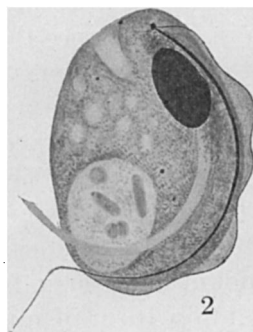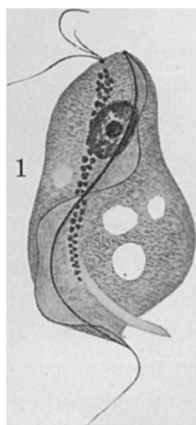
of the flagellates when stained on the slide present a lateral aspect as shown in Fig. 2, since they fall over on to their side in the drying out of the film.

But the appearance of the flagellate as described above does not endure for very long, simply because the trophozoite stage itself does not endure. The development of the trophozoite marks the period of youth, and when the organism has sufficiently fed on bacteria and cocci, and obtained a sufficient amount of reserve food, it passes on either into division or into a form of autogamous reproduction by which the flagellate population is increased at a rapid rate.

In the case of division, the process seems to be for the most part longitudinal. The first indication of it is to be seen in the blepharoplast-complex and in the nucleus. From each new blepharoplast there appears to grow out a new chromatic line, extending more or less parallel to the old line. From these new lines the new undulating membranes appear to arise. The writer has not been able to observe the division-stages of the flagella, although stages have been seen in which new flagella are present in connection with each new blepharoplast. Neither has it been possible to follow the changes in the axostyle. As to the chromatic blocks, these also seem to disappear and are probably formed anew in the daughter cells.

But reproduction by division, though occurring commonly in the intestinal content, is probably not the chief method of reproduction. At all times, though at some times more markedly than at others, the flagellates enter into a course of autogamous reproduction in which several daughter cells are formed out of a single mother cell. This interesting process can be followed in considerable detail by means of suitably stained smear preparations.

The first step in this process is the "rounding-off" of the previously elongate or crescentic trophozoite after it has reached maturity. If the body-form was crescentic there occurs a filling-out of the concave surface so that at first a full oval shape is produced; later the organism becomes spherical. This rounding-off process, which is

usually accompanied by some increase in size, is marked
by important changes in the structures alluded to above.
These may be considered in some detail first with ref-
erence to the external features.

Perhaps the most noteworthy change, aside from the
assumption of a spherical shape, involves the chromatic
line. This gives the appearance of lengthening until it
forms a hoop almost completely encircling the organism
(Fig. 4). It is common to see the ends of the line occupy-
ing positions less than 45 degrees apart as measured on
the circumference of the spherical flagellate. At the same
time the flagella have been lost and the undulatory' mem-
brane has decreased in size, though it follows approxi-
mately that part of the circumference corresponding to
the chromatic line. Of course its functioning has been
proportionately reduced and although its undulatory mo-
tion may continue, this movement fails to cause progres-
sive movement of the flagellate, but brings about a slow
rotation of the organism in the same position. Occa-
sionally this movement may be assisted by a single an-
terior flagellum or a remnant of one which remains after
the others have disappeared. In the final stage all trace
of the cytostome is lost, and in fresh preparations the or-
ganism appears as a ball of fairly homogeneous fluid, sur-
rounded by a granular cytoplasm containing the nucleus
(Fig. 5).

But more interesting are the changes that have been
occurring in the internal structures, as revealed by stained
preparations. The alterations in the food vacuole are
possibly the most significant. In the trophozoite stage
the vacuole was made up of one or more spaces represent-
ing probably not more than one eighth to one tenth of the
organism (Fig. 2). As the rounding-off process proceeds,
the vacuole increases in size until it occupies the greater
part of the ventral portion of the flagellate (Fig. 4). It
begins to crowd the cytoplasm against the dorsal wall,
and in this area lies the nucleus, which, as a result of
pressure, becomes somewhat flattened. At the same time
the "line of blocks" and the axostyle, which gives the

appearance of passing through the food vacuole, begin to degenerate and eventually both disappear. The chromatic line endures for a longer period, however, and remnants of it may be seen for some time after the ''line of blocks,'' axostyle and undulatory membrane have vanished. The blepharoplast also can be detected as long as the remnants of the chromatic line are visible (Figs. 6, 7). This increase in the size of the food vacuole seems to be due, partly at least, to the taking-in of fluid, since while this process is occurring the flagellate is increasing in size and becoming more plastic in the constitution of its protoplasm.

The food vacuole has now increased in size to represent the greater part of the flagellate cell and is surrounded by a crescentic ring or layer of cytoplasm seemingly much reduced in amount (Fig. 5). From this time on the most important changes concern the nucleus. This is now flattened or sometimes flask-shaped, and soon divides into two equal portions which travel through the region of cytoplasm to take positions at opposite sides of the ball of reserve substance (food vacuole). Here each experiences a further division resulting in the production of four daughter nuclei (Figs. 7, 8). These apparently may divide again until either eight or sixteen daughter nuclei are formed occupying positions about the periphery of the cell. Frequently smaller portions of nuclear substance are to be seen in the cytoplasm following the first nuclear division and it is probable that these represent reduction bodies (Fig. 8), although the writer has not observed them in the course of formation. About the daughter nuclei there seems to gather by slow degrees a layer of cytoplasm and eventually they break out of their peripheral ring of maternal cytoplasm to enter the ball of reserve substance occupying the center of the cell (Fig. 10). This is gradually consumed by the young organisms which slowly take on an elongated shape. During this time the cyst wall which had formed about the mother cell has been weakening and finally the young organisms break out of the mother cell and appear as the youngest

trophozoites measuring from 4 to 5 μ in length and about 3 μ in breadth, equipped with anterior flagella at least, and possessing a relatively large nucleus and minute blepharoplast. The other organelles characteristic of the mature trophozoites appear to develop by degrees as the trophozoite increases in size.

These, then, are the two chief methods of reproduction. Ordinarily the course is very simple, but from a study of both fresh and stained material it is clear that several complicating factors may enter. For instance there is evidence that conjugation may occur, not only between two individuals but perhaps between three or four. This process is aided by the extrusion of a viscid membrane by those organisms that have rounded-off. This naturally helps to cause the individuals to adhere together. After conjugation this viscid membrane appears to harden into a protective cyst wall. Usually the size of the single cyst is about 10 to 12 μ, but in the "fused" or conjugated forms the diameter may reach 20 to 30 μ as seen in fresh preparations. It is also clear that the "double" and "triple" cysts sometimes seen may represent a division of the original cyst, whereupon each daughter cyst continues independently the production of daughter cells by the usual method, described above.

Reproducing by the methods described above, *Trichomonas* ordinarily lives in the intestinal tract and causes no recognizable injury to the host. It has never been regarded as other than a harmless commensal. Recent studies[2] have demonstrated, however, that, upon occasion, this flagellate may depart from its usual mode of life, may penetrate the tissues of its host and cause fatal lesions, not only in the walls of the intestinal tract, but in the liver as well. It is especially this assumption of a pathogenic rôle, this sudden adaptation to a new manner of life, together with the morphological changes that accompany it, that constitute perhaps the most interesting phase of the life of *Trichomonas*. First, how does it happen that the flagellate gets started on its tissue-despoiling career?

[2] Rhode Island Agricultural Experiment Station, Bul. 166, 1916.

What is the first stimulus that creates out of a commonly law-abiding protozoan, an invader that has no equal among protozoan forms in the rapidity and completeness with which it carries on its ravages in the intestinal tissues?

This is a difficult question, and one which can not be answered with any degree of finality at the present time. The facts of the matter are these: The manifestation of the disease, as it appears for instance in the so-called blackhead of turkeys, is invariably preceded by a diarrheal condition in which the flagellates appear in increasing numbers as the course of the disease advances. Finally they appear, not only in the liquid cecal content, but in the very depths of the cecal tubules or crypts; and finally in the tissues behind the epithelial wall. From this position, by a process of autogamous reproduction, the invasion of the mucosa, submucosa, muscularis mucosæ and even the muscular layers, goes on rapidly; and eventually the whole cecal wall is crowded with the parasites. Secondary bacterial infections may intervene and the results are almost invariably fatal. The question now arises: Are these countless flagellates, present in the liquid cecal contents at the beginning of the attack, the *cause* or the *result* of the diarrheal condition? Clinical evidence, which can not now be presented in detail, seems to indicate that the latter circumstance is the actuality: that the diarrhea is the primary condition and the increase in the number of parasites the secondary. To explain the ''first cause'' of the disease, then, one must explain the cause of the diarrheal condition; and this, of course, is likely to prove in itself, a complex problem, but seems to lead back to certain circumstances related to the nature of the food materials and their assimilation, lying outside the province of the present paper.

For a long time it was not clear how, after their rapid multiplication in the intestinal content, the parasites were able to penetrate the epithelial wall and reach the subepithelial tissues. Recent studies[3] have shown the rôle

[3] Rhode Island Agricultural Experiment Station, Bul. 168, November, 1916.

played by the goblet or chalice cells of the crypts of Lieberkühn in this respect. *Trichomonas,* after congregating in vast numbers in the fundi of the crypts, with a consequent bulging of their walls forces its way into the goblet cells. It is not deterred by the nucleus or the cell wall at the basement end, but throws the former out of place and breaks through the latter to assume a position beneath the epithelium of the crypt. The wall having been ruptured and an avenue created to the deeper tissues, other flagellates follow by the same path until many are present between the epithelium and the basement membrane. But *Trichomonas* does not halt here. It is now filled with the spirit of the invasion and quickly pushes through the basement membrane into the loose connective tissue of the mucosa. This tissue is speedily overrun by the advancing hosts, the barrier of the muscularis mucosæ is passed and the entire submucosa exposed to the ravages of the parasite.

It is here that we recognize *Trichomonas* in a new rôle. Having experienced its first taste of blood its whole nature is changed; it becomes another animal, raging through the tissues and impeded by no protective action that the host organism is able to muster to the defense. Here then we must recognize *Trichomonas* as a cell parasite, an organism that has the power to actively invade living cells and to bring about their destruction. One may remark that the type of cell invaded is highly specialized type, and one that, by its nature, is more or less open to invasion. But the fact remains that *host cells are invaded,* and actively invaded; and in this circumstance we can detect, in the behavior of *Trichomonas,* a foreshadowing of those cell-invading activities regarded as characteristic of the sporozoa.

But of course the host-organism must put up some defense, and sometimes a very vigorous defense is offered, chiefly by means of its batteries of endothelial and other phagocytic cells. These come out in numbers to meet the invaders and as a result many of the flagellates are engulfed, either by single endothelial cells or in giant cells.

But the curious part of this circumstance is that the en-
gulfing of the parasites seems to be of slight avail in re-
tarding the invasion; and, in many instances without ap-
preciable detrimental effect upon the parasites engulfed.
From observations on the staining reactions and on the
morphological features of the ingested parasites there is
good evidence that *Trichomonas* is not disintegrated by
the process; and much less killed outright. It shows a
marked resistance to the plasm of the endothelial cells,
within which it frequently appears that development may
proceed, and from which a new generation of flagellates
may break out to continue the course of infection. This
would imply that the parasites, once engulfed, are able
to make use of the plasm of the endothelial cell as food.
And some evidence actually seems to support the view
that the parasites fare better in the endothelial cells than
they do without. In any region of invaded tissue the ma-
jority of the organisms are present within the engulfing
cells. If these views should prove valid it must be ad-
mitted that a curious situation is produced: the parasites,
to survive, must be ingested by the defensive cells, while
these phagocytic agents in carrying out their normal de-
fensive function, are favoring the growth and activity of
the invaders. Of course the residence of *Trichomonas*
within the endothelial cells is purely a passive cell-para-
sitism, although the penetration of the goblet cells is an
act of active cell-parasitism. But when we regard both
together, the matter is of considerable interest in its bear-
ing upon the origin of the sporozoa, cell parasites most
exclusively. From such elementary invasive power and
from such primitive toleration of unfavorable host-cell
influences as we see in *Trichomonas,* it is easy to imagine
how the most effective stages of sporozoan parasitism
may have evolved. It is a beginning of that marked
adaptability of form and of physiological organization
which lies at the base of all pure parasitism as it occurs
in the higher orders of the protozoa.

Another noteworthy feature in the life of *Trichomonas,*
and one which again serves to connect the organism with

the accepted type of sporozoan parasitism deals with the manner of obtaining its food. It has already been pointed out that when the trophozoites are developing in the intestinal contents they ingest large numbers of bacteria; whether, at this time, osmosis plays any part in cell nutrition is a question. . When *Trichomonas* has entered the deeper tissues, however, the situation is different, since there are ordinarily few bacteria in these regions. Here it seems that nutrition by osmosis must play an important rôle in supporting the life of the rapidly multiplying organisms. It thus appears that *Trichomonas* is sufficiently adaptive to new conditions of existence in the tissues to substitute an osmotic method of nutrition for the ingestive. This nutrition by osmosis it will be at once recognized is one of the characteristic features of the sporozoa, and here again is to be seen a link connecting these two protozoan types.

But there is another point of interest involved in this change in the manner of nutrition when *Trichomonas* enters upon its tissue despoiling career, and this concerns the influence of the manner of nutrition upon some of the morphological features of the parasites.

In regarding the appearance of the flagellates preceding their invasion of the tissues, and after they have gained a foothold in the submucosa, a marked difference is to be observed. This has already been mentioned and may be so great as to deceive one into the belief that the parasites which are found in the intact crypts and which penetrate the epithelial wall, are not identical in nature with the organisms occurring in the deeper tissues. It is this difference which has led some writers to believe that we are dealing with two different protozoan forms. The difference lies primarily in the following circumstance: In the cecal content the flagellates are represented by two forms, the motile trophozoite and the encysted organism. In the case of the latter, one can usually observe clearly the large ball of reserve-substance, and the relatively large daughter nuclei. When developing in the tissues, on the other hand, although the motile forms can be recognized

without difficulty and although the sporulating forms, characterized by the presence of the daughter nuclei, are also observable, both of these are relatively uncommon, and the stage which shows the well rounded ball of reserve-substance (''Reservestoffsballen'') is seldom met with. How can these phenomena be explained?

The writer has introduced this point in connection with the discussion of the methods of nutrition of the flagellates, simply because it seems possible that the morphological differences alluded to above are conditioned by the nature of the food supply. The writer has already traced the changes which the food vacuole of *Trichomonas* undergoes during the process of encystment. It was shown that there is a direct transformation from the food vacuole of the trophozoite, laden with bacteria and cocci, to the ball of reserve-substance which eventually crowds out the nucleus, chromatic line and line of blocks from the inner part of the cell and may possibly absorb the axostyle. Finally it comes to lie as a mass of varying size with respect to the cell, in the center, or slightly to the ventral side of the organism. Its staining qualities suggest a glycogen-like substance, and its density appears to vary with the stage of digestion of the food substances which are to serve the young daughter cells.

As stated above, this well defined reserve-substance mass is seldom observed, at least well developed, in the flagellates located deep in the tissues where the evidence favors a view of nutrition by osmosis. The question is therefore raised: Can it not be that the marked difference between the appearance of the flagellates in the tissues and in the cecal content is dependent directly upon the nature of the store of reserve food; and thus indirectly upon the manner of nutrition. This view is in agreement with the general observation that protozoa that subsist by osmosis seldom manifest either food vacuoles or definitely segregated bodies of reserve food substance.

In just what way the presence or absence of a ball of reserve-substance would explain all the differences observed in the parasites in and out of the tissues it is diffi-

cult to say. That its absence would determine a more homogeneous cytoplasm at all stages of growth is, of course, obvious, but its effect upon the cell structures such as chromatic line, blocks and axostyle, and upon the relative size and distribution of the daughter nuclei is still not clear. It can scarcely be wondered at, however, that such a radical change in the manner of nutrition of a parasite would be accompanied by alterations of some developmental significance.

Upon superficial observation it appears that, in a parasitism of this sort, when the organisms are driving ever deeper into the tissues, one of the essential features of complete parasitic activity is absent, namely, the ability to escape from the tissues and to secure a position by virtue of which the parasite can insure the possibility of reaching other hosts. Without this possibility provided for, no parasitism can be called complete. Although, in the case before us, many of the parasites are so buried in the tissues, a study of the trend of the infective process as a whole has revealed a means by which the organisms return to the cecal content after their invasive career has ended. This is by spreading downward and inward through the reticular tissue of the cores of the villi and pushing the epithelium off of the villus tips. Behind the epithelial wall at these points the parasites congregate in vast numbers until finally the epithelium breaks and liberates the flagellates into the cecal contents. That this process of escape from the tissues takes place only over certain areas of the intestinal wall is apparent; but the fact that it occurs at all is sufficient evidence to indicate that *Trichomonas* is not wholly lacking in this essential element of successful parasitism.

And finally we find in the case of *Trichomonas* one more lesson, and this is one for the etiologist, this being of course any one who concerns himself seriously with disease etiology. This important person, confronted with a disease of unknown cause, busily sets about to discover the germ; and having found the germ, he as busily engages himself in ascertaining means and measures

whereby the germ may be avoided by all susceptible folk.
We are warned to avoid the places where the germ lurks,
to boil our drinking water and to put cotton in our noses;
and of course this has been of immense value in prevent-
ing infection in the case of many communicable diseases.
But this conception of escaping the germ, a procedure
still by force of habit widely applied, unfortunately does
not work out successfully in all cases, simply because we
have at last found that the germ is not always escapable.
It may be right with us day and night; and whether we
succumb to an eventual invasion depends not upon our
side-stepping the organism, but upon our maintaining cer-
tain of the body defenses at the proper level of efficient
working.   The case of *Trichomonas* in its proper host is
an instance.   For twenty years (under other names) it has
been consistently avoided and wholesomely feared by in-
telligent turkey raisers.   Five hundred regulations more
or less have been directed against it; and now we find that
it is always there and always will be there.   To keep it in
an amicable state, to deter it from making destructive
excursions into the tissues, all that is required is to main-
tain a normal and hygienic condition of the intestinal
tract, whatever this may mean; this alone appears to be
sufficient.   Thus, although no other intestinal protozoan
is able to exert, in a brief time, a greater destructive
activity than *Trichomonas* when properly aroused, still
we are far from justified in placing its name upon the
blacklist of unqualifiedly pathogenic types which are, by
both heredity and training, trouble-makers.   On the other
hand we can not continue to place this flagellate in that
sainthood of parasites, the ''harmless commensals,'' since,
upon occasion, it may be far from harmless.   *Trichomonas*
must now be registered as a *facultative parasite,* which
offers a wealth of interesting subject-matter for research
covering several fields of biological study.